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(54) **Fermentation apparatus**

(57) Apparatus for use in fermentation processes, in cell culture and in processes for solubilising polysaccharides comprises a primary chamber (18) for holding liquid such as a fermentable feedstock, a nutrient medium or a chemical pre-treatment agent, and cylindrical secondary chambers (10) inside the primary chamber, each secondary chamber having a permeable wall (24), and means such as rollers (6) for rotating the secondary chambers inside the primary chamber. Support structures such as concentric cylindrical smooth or corrugated sheets (31, 32; 33, 34) may be mounted inside the secondary chamber. The apparatus may be used for the production of microbial biomass by growing a microorganism on a substrate in the secondary chamber. The apparatus may also be used for production of alcohol, enzymes, cultured plant or animal cells, antibiotics, products of genetic engineering or for solubilising polysaccharides.

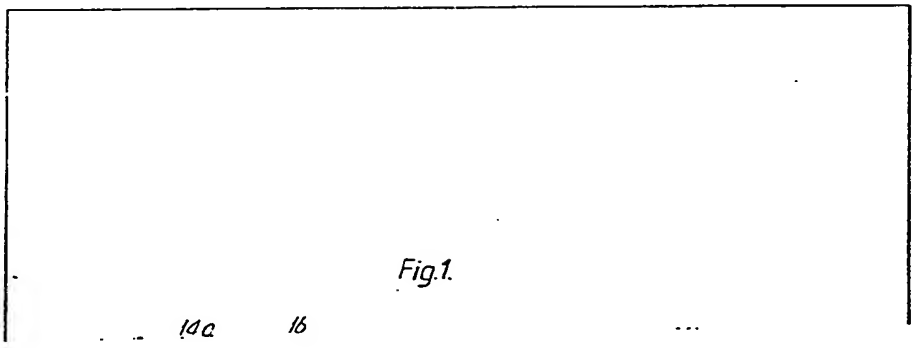

Fig.1.

ERRATUM

SPECIFICATION NO 2097817A

Front page, Heading (72), Inventors *delete* whole lines *insert* Patrick Prendergast Edward John Prendergast Elizabeth Shanahan

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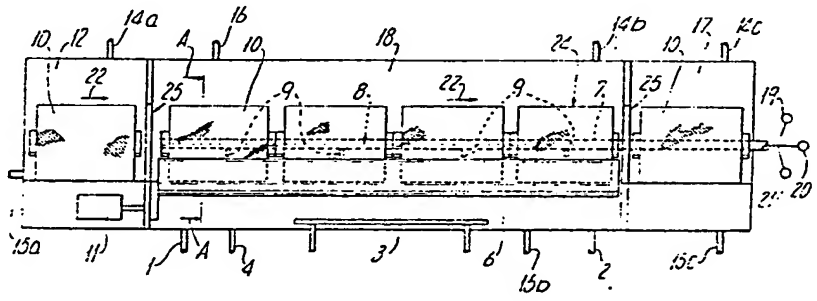
Fig.1.



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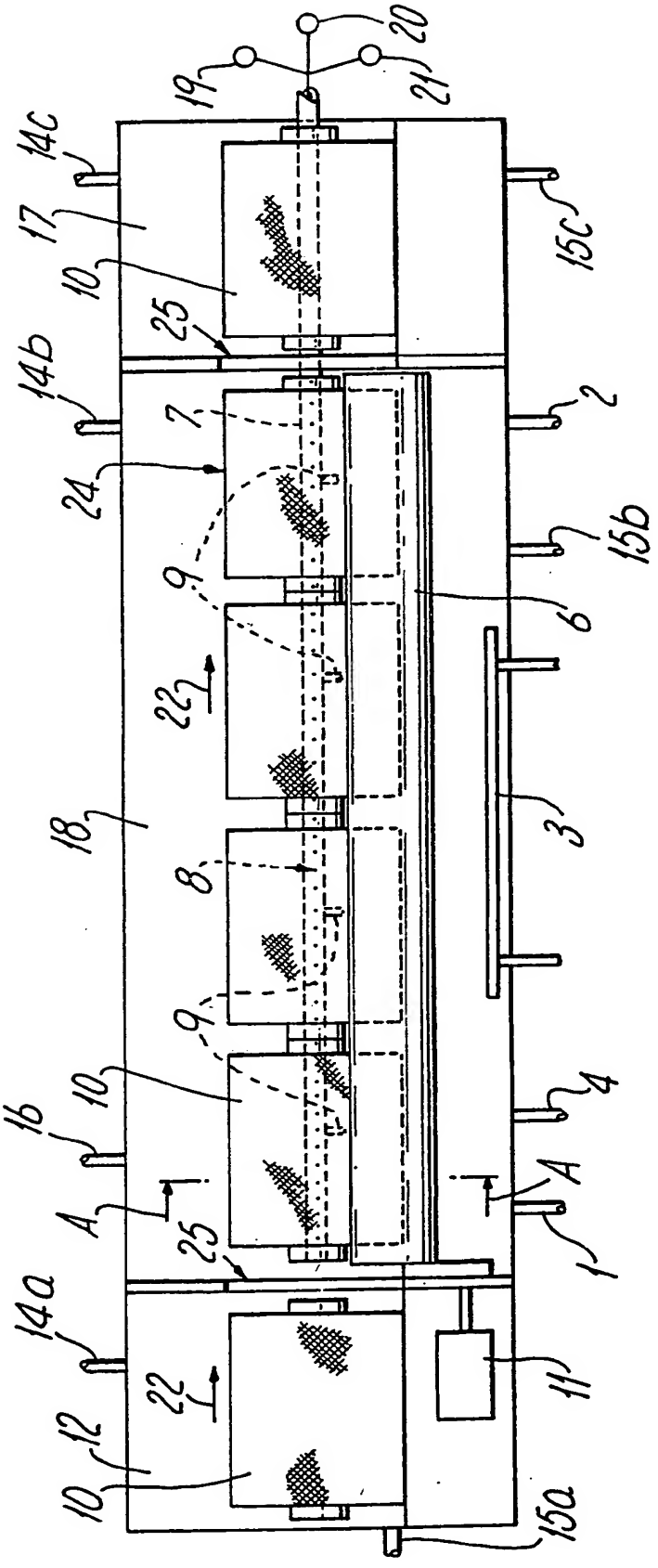

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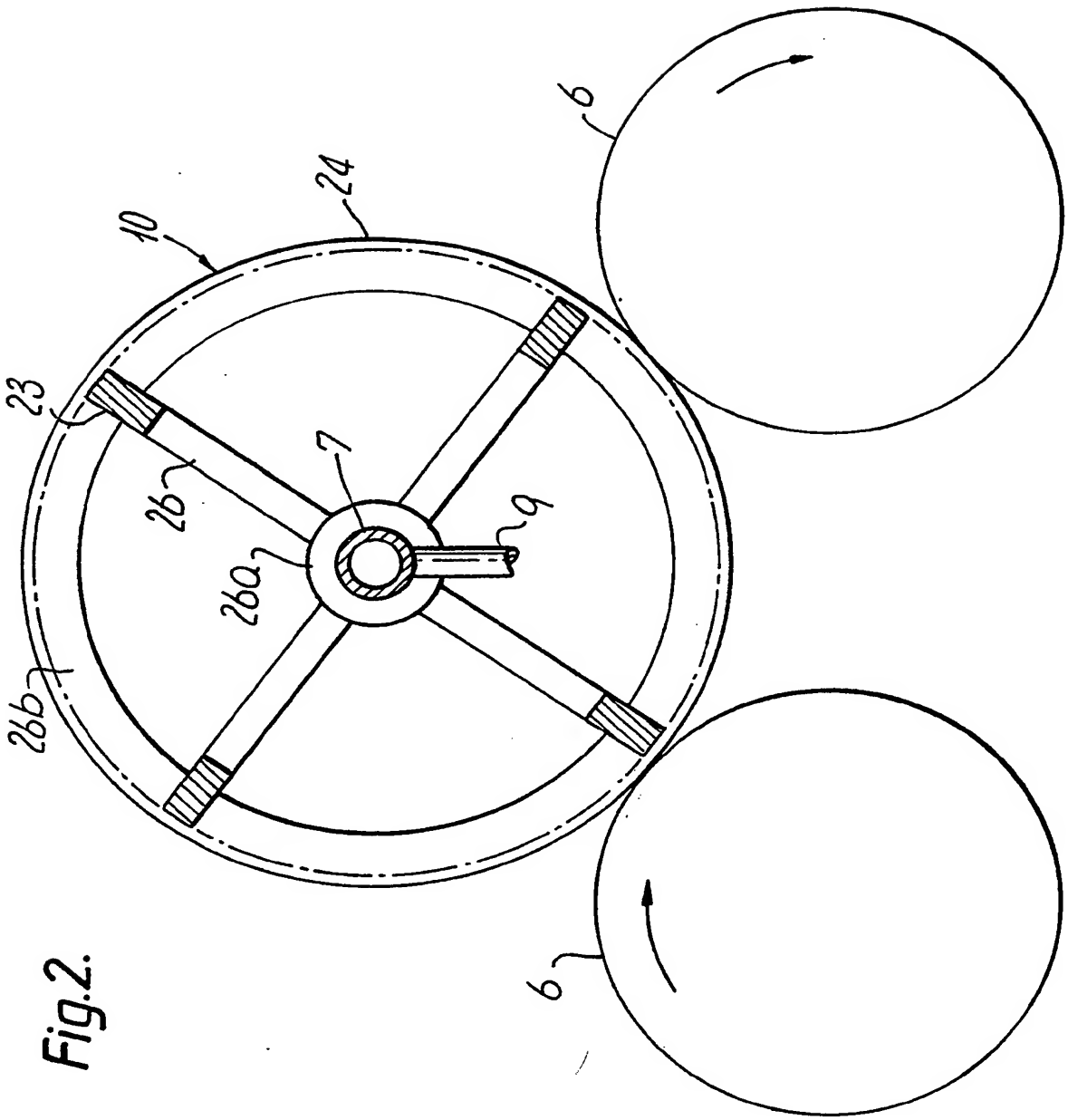

Fig.2.

Fig.3.

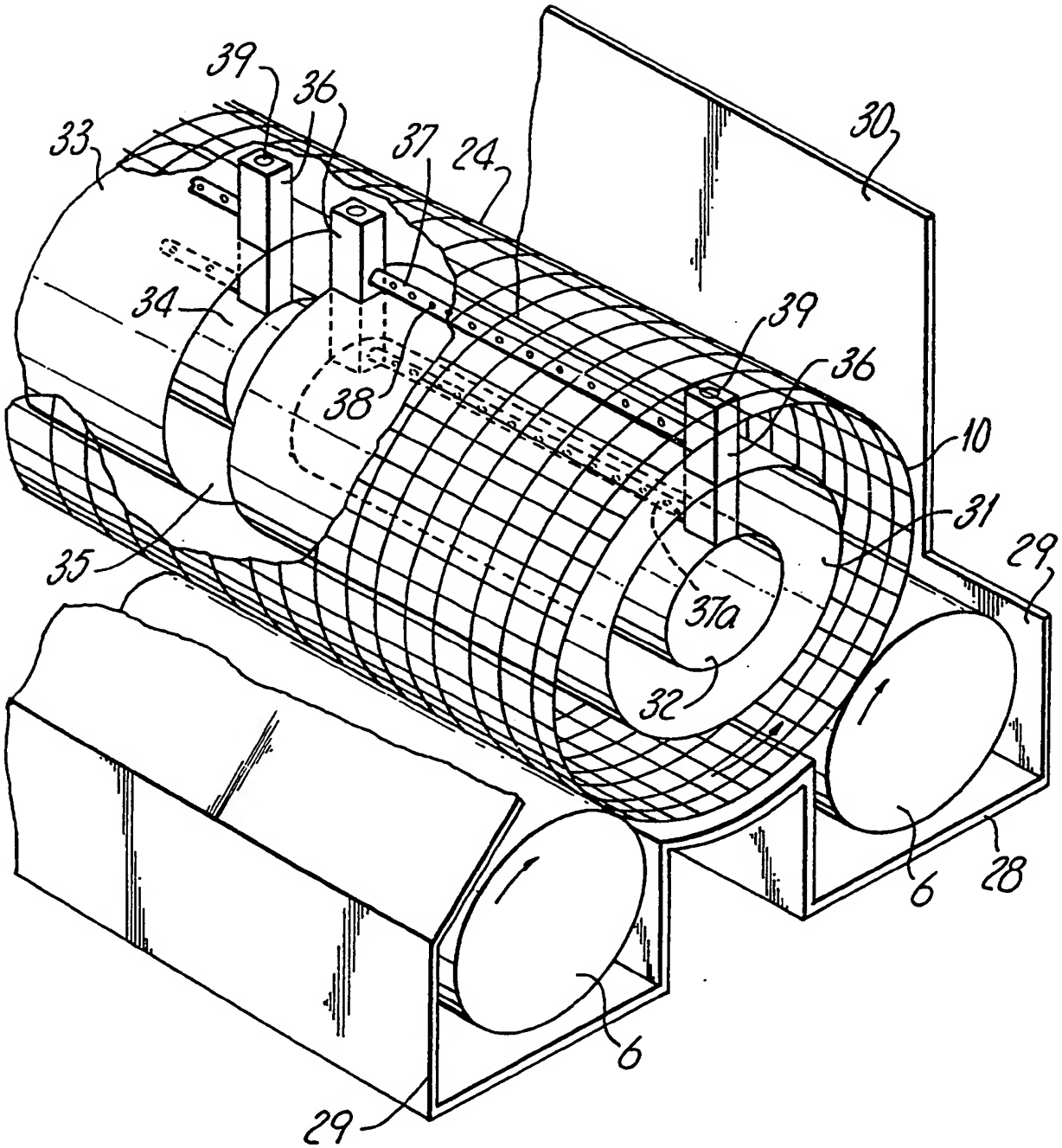

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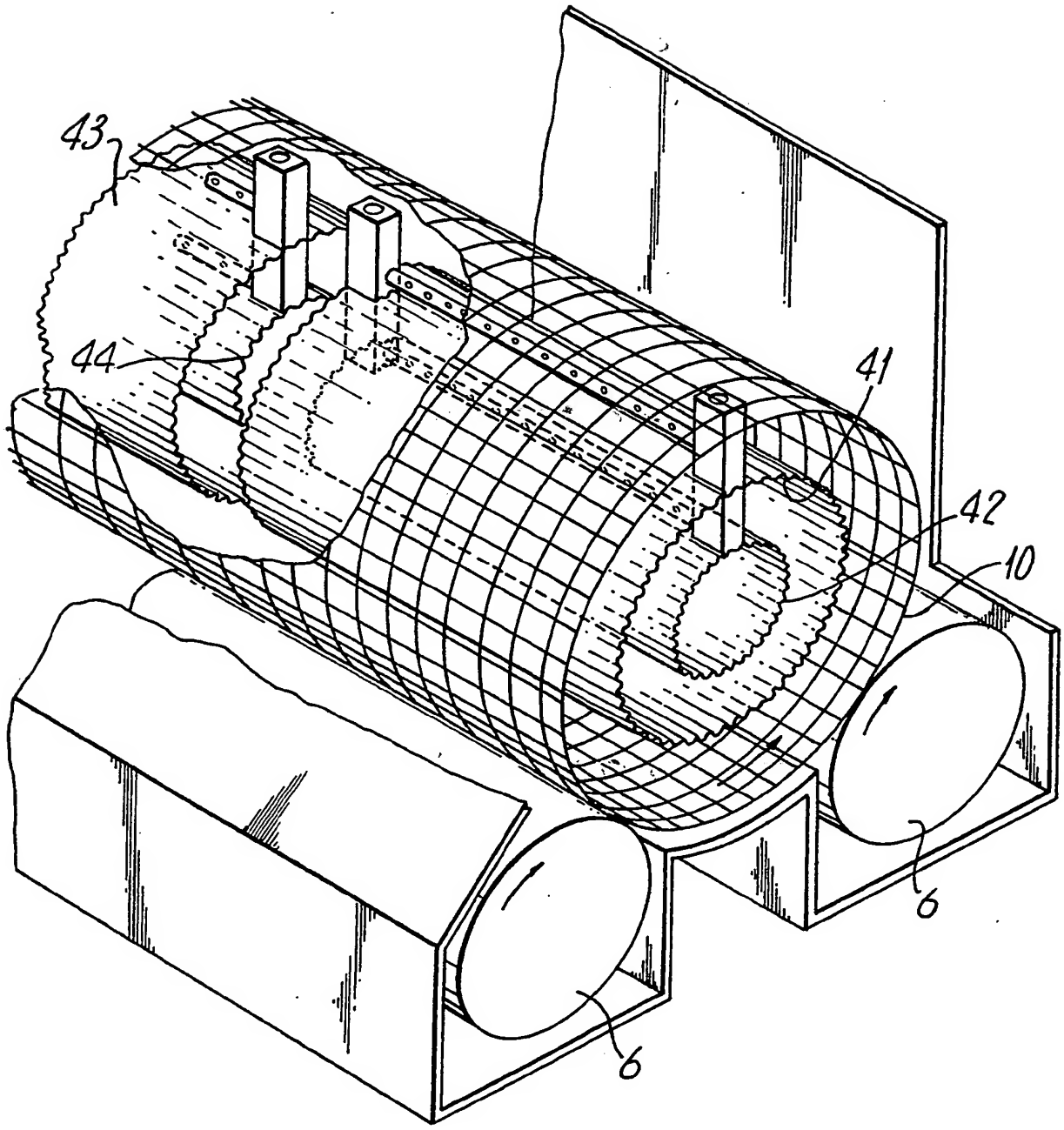
Fig.4.



SPECIFICATION

Apparatus for use in fermentation processes

This invention relates to apparatus for use in fermentation processes, in cell culture and in processes for solubilising polysaccharides. The invention also relates to fermentation and other processes carried out in the apparatus.

Fermentation processes are widely used in industry, for example in the production of alcohol and pharmaceutical products, such as antibiotics. The field of "genetic engineering" utilises fermentation processes for the bacterial production of products such as interferon. Experimental work is also being carried out on the use of fermentation processes for the bioconversion or "upgrading" of agricultural waste materials into proteinaceous feed products (see U.K. Patent Application No. 2,041,403A and "The Waterloo Process for SCP Production from Waste Biomass" by M. Moo Young, A. J. Daugulis, D. S. Chahal and D. G. Macdonald, *Process Biochemistry*, October 1979, 38—40). The highest growth rate is achieved with liquid fermentation, for example in a stirred-tank fermenter, but it is difficult to harvest the product. Experimental work is also being carried out with tray fermenters, which facilitate harvesting but which result in lower growth rates and problems of nutrient exhaustion. A tubular fermenter incorporating wall scrapers has also been suggested (British Patent Specification No. 1,499,410).

The present invention provides apparatus for use in fermentation processes, in cell culture, and processes for solubilising polysaccharides, comprising a primary chamber for holding liquid, a secondary support structure adapted to be located inside the primary chamber, said secondary support structure in conjunction with a permeable wall therefor defining a secondary chamber, and means for rotating the secondary support structure inside the primary chamber.

Preferably, the secondary chamber is cylindrical and the rotation means is adapted to rotate the secondary chamber about its axis.

In a particularly preferred feature, the means for rotating the secondary chamber comprises a bed of rollers in the primary chamber having their axes parallel to that of the secondary chamber.

In one embodiment, the apparatus further comprises means for conveying a plurality of secondary chambers through the primary chamber.

In another aspect, the invention provides a fermentation process wherein a microorganism or enzyme is supported on a secondary support structure or is contained in a secondary chamber, said secondary chamber having a wall which is permeable to a liquid nutrient medium for the microorganism or to a liquid feedstock fermentable by the enzyme, and the secondary support structure or chamber is rotatably located during the fermentation inside a primary chamber, through which primary chamber a liquid nutrient medium for the microorganism or a liquid feedstock fermentable by the enzyme is circulated.

In a preferred feature the invention provides a fermentation process which comprises growing a microorganism on a substrate which is utilized by the microorganism for growth, in the presence of a nutrient medium for the microorganism, wherein the substrate is contained in a secondary chamber having a wall which is permeable to the nutrient medium, and the secondary chamber is rotatably located during the fermentation inside a primary chamber.

In the following description, the term "nutrient medium" is used to describe the circulating liquid. However it will be understood that in the case of fermentation using an enzyme (not in the presence of a microorganism), the circulating liquid will be a feedstock which is fermentable by the enzyme, e.g. a glucose solution.

When a substrate is present in the growing chamber (the secondary chamber), the permeable wall of the growing chamber is chosen so as to permit the nutrient medium to pass through it but so that the substrate does not pass out through it. The substrate may be in the form of a liquid, a slurry, a paste or in the form of flowable solid particles. If the fermentation product is to be harvested as a solid, then the product also should not pass through the permeable wall. Varying sizes of pore may be chosen and e.g. gauze, dialysis membranes, etc., may be suitable.

The fermentation may be aerobic or anaerobic. The apparatus of the invention is particularly suited to an aerobic fermentation for which air is supplied to the growing chamber directly or in the liquid medium.

The primary chamber may be partially or completely filled with liquid medium. The growing chamber may be moved through the primary chamber in such a manner that the growing chamber is brought above the level of the medium before the growing chamber leaves the primary chamber.

The apparatus and process of the invention are particularly adapted for production of microbial biomass, more particularly by the mass microbial cultivation of the fungus *chaetomium cellulolyticum* which is described in U.K. Patent Application No. 2,041,403A. The microorganism microorganism may suitably be another cellulolytic microorganism, for example the mould Trichoderme Veride. The process can be carried out on a polysaccharide substrate, particularly cellulose. The process may also produce an extracellular enzyme which acts on the cellulose. A substrate may be in any suitable solid form, e.g. particles, chopped or unchopped strands, etc. The substrate may be starch, a carbohydrate, lignin or a hydrocarbon, for example.

The process for production of microbial biomass is most suitably carried out on waste products of agriculture, forestry or industry, more particularly crop residues such as straw, fruit processing waste or wood or other sawmill residues. The waste products may be pretreated

chemically or physically.

The process is particularly adapted for the production of proteinaceous animal feed which is harvested in solid form from the growing chamber. However the end product may be, for example, other fungi, alcohol, an enzyme, cultured plant or animal cells, an antibiotic or a product of genetic engineering such as interferon. The end product may collect in the nutrient medium and a soluble end product can be extracted from the spent medium by any suitable means. Alternatively the product may be grown on a support structure in the growing chamber. The end product may be a gas, such as methane or hydrogen, which can be trapped above the growing chamber.

If desired, an enzyme can be used in the nutrient medium to chemically alter a substrate in the growing chamber.

The secondary chamber may be any suitable cylindrical container with permeable wall which can be located in the medium inside the primary chamber. The secondary chamber may suitably be a drum shaped framework to which a cylindrical permeable wall can be attached. The framework may include a cylindrical wall of coarse mesh, over which a more-finely pored gauze or dialysis membrane may be attached.

The contents of the secondary chamber should be agitated and this is achieved by rotating the secondary chamber e.g. by mounting the chamber on rollers or on an axle. For an aerobic fermentation, air can be pumped into the interior of the secondary chamber e.g. through the axle or other pipe passing through the primary chamber and entering the secondary chamber.

In a preferred manner of carrying out the process for growing a microorganism on a substrate in accordance with the invention the substrate is initially sterilised *in situ* in the growing chamber e.g. by placing the growing chamber in an autoclave. For example, in the case of straw, sterilisation can be carried out with steam at 16 p.s.i. for about 15 minutes. Alternatively sterilisation of the chambers and the substrate can be achieved by the use of sterilizing agents in gaseous or liquid form e.g. hydrogen fluoride or ethylene oxide gas, chloroform, ethanol or the like in liquid form. The substrate is then innoculated with the desired organism or organisms. Following this, both the primary chamber and the growing chamber located inside it are filled with a liquid nutrient medium appropriate for the organism or organisms being used so that the substrate is immersed in the medium. The medium is circulated through both chambers from a reservoir, in which the medium can be reconstituted or replenished as required. Agitation and forced aeration of the substrate is carried out in the growing chamber during the course of the fermentation. The pH of the medium is monitored, and the temperature of both chambers is maintained at the desired level by means of a thermostat and heating source which is preferably incorporated into the medium circulation system. For example, when using the fungus, *chaetomium cellulolyticum*, the pH should be around 5.5 and the temperature of the medium and/or the growing chamber should be about 37°C.

The fermentation product or upgraded product builds up in the growing chamber. The process is allowed to continue until the desired concentration of end product is reached. Then the liquid medium is removed, e.g. by pumping or draining it out of the primary chamber, or the growing chamber is removed from the primary chamber. The product is allowed to dry e.g. with the help of hot air. The product is then harvested in a semi-solid or moist condition. The process is adapted for operation on a continuous basis.

Some embodiments of apparatus in accordance with the invention will be described below by way of illustration with reference to the accompanying drawings, in which:

Figure 1 is a schematic elevation of one embodiment of the apparatus.

Figure 2 is an enlarged schematic cross section on the line A—A in figure 1.

Figure 3 is a projection of part of a secondary chamber for a second embodiment of the invention, showing also part of the primary chamber.

Figure 4 is a projection of part of a secondary chamber for a third embodiment of the invention.

The apparatus shown in Figures 1 and 2 uses a plurality of cylindrical growing chambers 10 (of which 6 are shown in Figure 1), each having a permeable or porous wall 24. Each growing chamber 10 passes first into an optional sterilization chamber 12. The loading of substrate into growing chamber 10 to the desired level can be performed either before or after entry of the growing chamber 10 into the sterilization chamber 12. Sterilization is effected by allowing steam to enter the sterilization chamber by inlet 15 and the steam is released after sterilization via exhaust outlet 14.

After sterilization the growing chamber 14 is displaced in the direction of arrow 22 through an opening 25 into the primary chamber 18. As illustrated, the primary chamber is long enough to house 4 growing chambers passing in sequence through it. Each growing chamber rests on a powered roller bed comprising two rollers 6 driven in the same direction by an external motor 11. Rotation of the rollers 6 causes rotation of the cylindrical growing chambers 10, causing agitation of the substrate inside the growing chambers. As shown in Figure 2, the growing chamber has radial baffle bars 23 spaced around its interior surface to disrupt the movement of the substrate. These radial baffles 23 form part of a drum-shaped framework formed by radial arms 26 at each end of the chamber, extending from a central collar 26a to a circumferential rim 26b, and the baffles 23 extending for the length of the chamber between respective parts of arms 26. The number of baffle bars can exceed four, if desired. The cylindrical wall 24, suitably of wire mesh, is applied around and supported by the baffles 23 and the rims 26b. A gauze or

membrane may then be attached over the mesh, and may be moulded into position on the mesh.

On entering the primary chamber 18, each growing chamber 10 fits over an air sparger rod 7 which passes axially through the growing chamber. The air sparger rod 7 also carries inoculation and sampling ducts 9. The substrate is inoculated with the required microorganism which is supplied by an inoculation pump through the inoculation duct 9. The nutrient medium is pumped into the primary chamber via medium inlet 1. The desired temperature for the fermentation is maintained within both chambers by heating unit 3 which is thermostatically controlled. pH is continuously monitored by probe 4. The air sparger rod 7 has orifices 8 through which air is pumped by pump 20 to aerate the substrate, which is being tumbled in the growing chamber.

The liquid medium is circulated through the primary chamber, being withdrawn via exit 2 and returned to a reservoir where the medium is replenished or reconstituted. The level of liquid medium in the chambers may vary depending upon the type of fermentation in progress. Samples of the contents of any growing chamber 10 may be removed at any time during the course of the fermentation by sampling pump 21 via sampling duct 9.

Effluent gas can be vented from the primary chamber via outlet 16. The primary chamber can be steam sterilized via inlet 15b and outlet 14b.

When the desired end-product concentration has been reached in growing chamber 10, it is displaced through exit 25 into a harvesting bay 17. The level of liquid medium in the primary chamber 18 must be reduced below the level of the entry and exit doors 25 prior to opening of these doors to permit a growing chamber 10 to enter or leave the primary chamber.

Instead of the air sparger rod 7 and ducts 9, a group of flexible tubes may be attached to each growing chamber, with different tubes allocated to air supply, inoculation and sampling. If desired the tubes may comprise or may be associated with means for forwarding the growing chambers through the primary chamber.

Preferably, the growing chambers are conveyed through the primary chambers on the roller bed, the forwarding movement being effected by successive growing chambers being pushed forwardly. The roller bed may incorporate helical forwarding means, if desired.

In a modified form of the apparatus, the roller bed is inclined upwardly from left to right in Figure 1 so that the growing chamber is displaced above the level of the liquid medium before it leaves the primary chamber. Drying of the product can then be carried out in the primary chamber.

While in the harvesting bay 17, the product can be sterilized by steam or other sterilizing agent via inlet 15c and outlet 14c. The product is allowed to dry and can then be harvested in a moist or dry condition, depending on the type of product required.

If the process of the present invention is carried out on a straw utilizing *chaetomium cellulolyticum* up to 50% W/V solids loading can be achieved (e.g. 50 gm straw per 100 ml nutrient medium) and an edible fungus product of 80% protein content (as a percentage of dry matter) can be obtained. If the product is harvested at about 16% protein, it can be fed direct to animals, or if it is harvested at higher protein concentrations it can be mixed with other feedstuffs.

The dual chamber process according to the present invention can be used to ferment or upgrade a higher substrate concentration per unit volume of liquid nutrient medium than other processes. It combines the high growth rate associated with submerged fermentation with the ability to harvest the end product in a concentrated moistened form, which has hitherto been possible only with tray fermentation methods, where growth rate is severely retarded. The dangers of oxygen limitation and "sporing", which can occur if the nutrient is exhausted in a tray fermentation method, is avoided. The time required for achieving the desired end product concentration is reduced as compared to other processes.

Figure 3 shows a special form of secondary chamber particularly adapted for the culture of plant or animal cells in monolayers on a solid base. The chamber has a cylindrical outer wall 24 of steel mesh over which a dialysis membrane, can be fitted and inside which are mounted concentric cylindrical sheet structures in pairs 31, 32; 33, 34. Each of the sheet structures extends in the axial direction for only part of the length of the chamber and gaps 35 are provided between successive pairs of sheet structures so that liquid medium can circulate freely between them and into the annular spaces between the sheets. For example, in a secondary chamber which is 2 ft (0.6 m) long, there may be three pairs of sheets, each about 7 inches (17.8 cm) long, with gaps 35 of about 1.5 inch (3.8 cm) between them.

Each pair of sheets is supported by radial mounting posts 36 at each end of the sheet. Between each pair of mounting posts there is a pipe 37 provided with apertures 38. A self-sealing inoculum port 39 in each of the posts communicates with the pipe 37. A second pipe 37a is arranged in the space between the sheets 31 and 32.

The primary chamber has a shaped floor 28 which lies close to the rollers 6 and extends up between the rollers 6 until it approaches the bottom of the secondary chamber 10, whose circumferential shape it follows. This moulded floor is useful to ensure that the minimum amount of nutrient medium is used, in cases where partial or fully submerged culture conditions are required, or when very little medium is needed, merely to keep a membrane wall of the secondary chamber moist. It is a particularly valuable feature when the nutrient medium is very costly. The side walls 29 of the primary chamber are also adjacent to the rollers and Figure 3 shows on one side a partition

30 which extends up to the height of the secondary chamber. This partition is used if more than one installation of roller beds with secondary chambers thereon is arranged inside a single primary chamber, so that the liquid medium is kept separate.

A suspension of the cells to be cultured is inoculated via the ports 39 and apertures 38, and forms a thin line of cells as a monolayer on each of the sheet cylinders 31, 32; 33, 34. A small quantity of nutrient medium is circulated in the primary chamber and it is just sufficient to keep the dialysis membrane moist. As cell culture proceeds, the cells divide and grow in a monolayer around the surfaces of the sheet cylinders, until they meet on the far side of each cylinder.

This kind of monolayer culture is particularly suitable for cell culture in genetic studies, or for production of viral vaccines, interferon and hormones, which require anchorage-dependent cells.

Figure 4 shows a secondary chamber similar to that of Figure 3 except that the concentric cylinders 41, 42; 43, 44 are corrugated and therefore have greater surface area than purely cylindrical sheets. This type of structure is particularly suitable for fermentation processes using enzymes or bacterial or fungal cells held in a matrix such as various resins, and gels particularly by ionic cross-linking of a charged polymer, e.g. alginate or entrapment gels, e.g. agar, collagen or polyacrylamide. The matrix can be applied on both internal and external surfaces of the cylinders. If the cylinders are of suitable plastics materials which carry electrostatic charge, the matrix may be held in position without assistance. Alternatively a fine gauze can be applied around the matrix.

The secondary chamber of Figure 5 is usually completely submerged in the feedstock or medium in the primary chamber. Cells (such as yeast) fixed in the matrix do not themselves grow and do not use up the nutrient to a substantial extent, so that a very high conversion ratios can be achieved in the fermentation process. For example, it has been found that the ratio of conversion of glucose to alcohol in the process using fixed yeast can be increased by a factor of 9.

For enzymatic and fixed cell processes, the medium can be aerated and then a sparger 7 is not necessary. In some instances, the permeable wall can be omitted.

In the chambers of both Figures 3 and 4, there may be more than two concentric cylinders between the axis and the circumference of the chamber.

The chambers of Figures 3 and 4 can be placed on a roller bed in the apparatus of Figure 1, omitting the air sparger rod 7 and inoculation ducts 9.

The chambers of Figures 3 and 4 have the same support structure of radial arms 26 and longitudinal bars 23 as that of Figure 2, but these are omitted in Figures 3 and 4 for clarity.

Each secondary chamber may have solid end walls e.g. metal plates attached to the radial arms 26.

This is usually necessary if a membrane in the form of a dialysis bag is being used over the cylindrical mesh or gauze. Alternatively the end walls of the secondary chambers may be formed of gauze.

A portion of the gauze can be removable to provide an aperture through which the secondary chamber can be loaded. The removable gauze portion can be put back and held in position by suitable clips.

Atomizer spray units may be incorporated into the primary chambers, normally above the level of the secondary chambers. When the apparatus is being used with a low level of medium in the primary chamber, the sprays can supply the medium to the secondary chambers and also maintain a humid atmosphere. Surplus medium collecting below the secondary chambers is recycled through the sprays.

A refrigeration system may be incorporated into the primary chamber. They may operate to reduce the temperature of the system to 10 degrees below zero (Celsius) when required, e.g. for storing enzymes at the end of a production run, or in the pretreatment of cellulose (described below).

When the apparatus is being used for growing a solid product (fungus), the permeable wall of the secondary chamber can be a wire gauze which is sufficiently fine to keep the solid inside while the chamber is rotating but which allows the medium to pass in and out.

For enzyme production and cell growth (e.g. for antibiotics), it is preferred to use a dialysis membrane applied over gauze. A certain volume of liquid nutrient medium is placed into the secondary chamber initially. Then as the growth proceeds, a nutrient salt can pass through the membrane by osmosis as the nutrient in the secondary chamber is consumed by the organism. There is no bulk transfer of water through the membrane and the volume of liquid in the secondary chamber stays substantially constant. The salt concentration outside the secondary chambers is kept slightly in excess, so as to maintain the osmotic gradient across the membrane.

When producing an enzyme in the secondary chamber, it will be retained inside the dialysis membrane.

If all of the secondary chambers in one primary chamber have the same inoculum and can be allowed to proceed on a full course of cell growth without interruption, a gauze with a relatively large pore size can be used. Although changes of pH occur during the growth cycle of plant or animal cells, it is satisfactory to allow the medium to circulate in all of the secondary chambers, if they are all at the same stage of the cycle. However if the various secondary chambers are at different stages in the growth cycle, a dialysis membrane is usually needed on each secondary chamber.

A line of secondary chambers in a primary

chamber can be operated as a closed system as regards recycling of nutrient medium, substrate inoculum, aeration, pH, mixing and all other parameters of growth, such that almost ideal plug-flow fermenting consitions are achieved for production of enzymes or antibiotics. If several primary chambers are operated side-by-side, each primary chamber containing secondary chambers at different stages of the growth cycle, substantially continuous production can be achieved without moving the secondary chamber through any of the primary chambers.

In processes involving transformation or conversions effected by enzymes, it is possible to locate different enzymes in successive secondary chambers along a single primary chamber, and to flow the medium through the primary chamber past the secondary chambers in succession. Thus progressive transformation of an initial substrate can be achieved.

In addition to its use in fermentation processes, the apparatus according to the invention finds use in the pretreatment of polysaccharides such as cellulose in substrates as described above to solubilise the polysaccharides to glucose and oligosaccharides. The cellulose material is carried in the secondary chamber in the manner described above, but the nutrient medium is replaced by a chemical pre-treatment agent, particularly hydrogen fluoride. A plastic gauze is used as the wall of the secondary chamber and the whole interior of the apparatus is spray coated with e.g. polyethylene to prevent attack by HF on metal. The liquid hydrogen fluoride (which can be anhydrous or contain up to 20% water) at temperatures in the range +15°C to −10°C solubilises cellulose to glucose which passes through the gauze and is removed from the system in the HF and separated by flash evaporation at low pressure. A residue of lignin is retained in the secondary chamber by the gauze, and this can be upgraded to protein by the process described above. The glucose can be fermented to alcohol, using a fixed enzyme, or can be used for growth of yeast cells or for tissue culture, suitably in the apparatus of the invention.

CLAIMS

1. Apparatus for use in fermentation processes, in cell culture, and processes for solubilising polysaccharides, comprising a primary chamber for holding liquid, a secondary support structure adapted to be located inside the primary chamber, said secondary support structure in conjunction with a permeable wall therefor defining a secondary chamber, and means for rotating the secondary support structure inside the primary chamber.

2. Apparatus according to claim 1 comprising a primary chamber for holding a liquid, a secondary chamber and a support structure therefor adapted to be located inside the primary chamber, the secondary chamber having a wall which is permeable to the liquid, and means for rotating the secondary chamber inside the primary chamber.

3. Apparatus according to claim 2, wherein the secondary chamber is cylindrical and the rotation means is adapted to rotate the secondary chamber about its axis.

4. Apparatus according to claim 3, wherein the means for rotating the secondary chamber comprises a bed of rollers in the primary chamber having their axes parallel to that of the secondary chamber.

5. Apparatus according to any of claims 2—4 which further comprises means for conveying a plurality of secondary chambers through the primary chamber.

6. Apparatus according to either of claims 3 and 4, wherein the secondary chamber comprises a support structure of radial arms at each end of the chamber and longitudinal bars extending between the free ends of corresponding radial arms, with a cylindrical permeable wall surrounding the longitudinal bars.

7. Apparatus according to claim 4, wherein the primary chamber has a floor which is shaped to fit closely to the rollers and the lower circumference of the secondary chambers.

8. Apparatus according to claim 1, wherein the support structure comprises a plurality of concentric cylindrical sheet surfaces.

9. Apparatus according to claim 8, wherein the cylindrical sheet surfaces are corrugated.

10. A fermentation process wherein a microorganism or enzyme is supported on a secondary support structure of apparatus according to claim 1 or is contained in a secondary chamber of apparatus according to claim 2, said secondary chamber having a wall which is permeable to a liquid nutrient medium for the microorganism or to a liquid feedstock fermentable by the enzyme, and the secondary support structure or chamber is located during the fermentation inside a primary chamber of apparatus according to claim 1 or 2, through which primary chamber a liquid nutrient medium for the microorganism or a liquid feedstock fermentable by the enzyme is circulated.

11. A fermentation process which comprises growing a microorganism on a substrate which is utilized by the microorganism for growth, in the presence of a nutrient medium for the microorganism, wherein the substrate is contained in a secondary chamber of apparatus according to claim 2, said secondary chamber having a wall which is permeable to the nutrient medium, and the secondary chamber is located during the fermentation inside a primary chamber of apparatus according to claim 2, through which primary chamber the nutrient medium is circulated.

12. A process of cell culture wherein plant or animal cells are grown on a support structure or in a secondary chamber of apparatus according to claim 1, said secondary chamber having a wall which is permeable to a liquid nutrient medium for cell growth, and the secondary support structure or chamber is located during the growing process

inside a primary chamber of apparatus according to claim 1 or 2, through which primary chamber a liquid nutrient medium for the cell growth is circulated.

13. A process according to claim 10, wherein the support structure comprises a plurality of concentric cylindrical sheet surfaces and the cells are grown in a monolayer from a line of cells placed parallel to the axis on each of said sheet surfaces.

14. A process of fermentation according to claim 10, wherein an enzyme or microorganism held in a matrix is supported on the secondary support structure.

15. A process according to claim 14, wherein the support structure comprises a plurality of concentric cylindrical corrugated sheet surfaces.

16. A process for solubilising polysaccharides wherein the polysaccharide is contained in a secondary chamber of apparatus according to claim 2, the secondary chamber having a wall which is permeable to a liquid chemical pre-treatment agent for solubilising the polysaccharide, but which does not permit the polysaccharide to pass through it, and the secondary chamber is located inside a primary chamber of apparatus according to claim 2, through which primary chamber a liquid chemical pre-treatment agent is circulated.

17. A process according to claim 16 wherein the pre-treatment agent is hydrogen fluoride, and the process is conducted at a temperature in the range from +15°C to −10°C.

18. Apparatus for use in fermentation, in cell culture, or in solubilising polysaccharides, substantially as described herein with reference to Figures 1 and 2 or 3 or 4 of the accompanying drawings.

19. A process of fermentation, of cell culture or of polysaccharide solubilisation, substantially as described herein.

20. Product of a process according to any of claims 10—17 or 19.

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